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Prostaglandins protect kidneys against ischemic and toxic injury by a cellular effect

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Prostaglandins protect kidneys against ischemic and toxic injury by a cellular effect. The ability of prostaglandins to protect the kidney against ischemic and toxic renal injury was evaluated by in vivo and in vitro models of renal ischemia. The prostaglandin E_1 analogue, misoprostol, was found to provide significant protection against ischemia-induced renal dysfunction in rats subjected to 40 minutes of renal artery occlusion. Misoprostol-treated rats had glomerular filtration rates almost threefold greater than control animals, although renal blood flow and renal vascular resistance were not significantly different. Improved tubular function was reflected in a lower fractional excretion of sodium and a higher urine-to-plasma creatinine ratio. Misoprostol also provided similar protection in a model of toxic renal injury produced by mercuric chloride. In an in vitro model employing primary cultures of proximal tubule epithelial cells subjected to hypoxia and reoxygenation, misoprostol limited cell death. Posthypoxic cells had apical membrane disruption and loss of microvilli when examined by transmission electron microscopy. These changes were not seen in misoprostol-treated cells. The "cytoprotective" effect was also produced by prostaglandin E_2 and prostacyclin. The ability of prostaglandin E to protect against toxic and ischemic renal injury did not appear to be due to an antioxidant effect because misoprostol did not limit lipid peroxidation in vivo and did not protect against oxidant injury by tert-butyl hydroperoxide in vitro. Although the exact mechanism of prostaglandin protection was not revealed, these studies demonstrate that prostaglandins protect renal tubule epithelial cells from hypoxic injury at the cellular level independent of hemodynamic factors or inflammatory responses. Such a "cytoprotective" effect of prostaglandins may be a generalized phenomenon since it has also been demonstrated in gastrointestinal epithelium.

Prostaglandins have numerous renal effects on hemodynamics, ion transport, and metabolism [1]. In addition, several investigators have reported beneficial actions of prostaglandin E or prostacyclin to limit ischemic or toxic renal injury [2–15]. In some studies prostaglandins increased both renal blood flow (RBF) and glomerular filtration rate (GFR), suggesting that the increase in RBF was responsible for the improved GFR. However, in other studies, exogenous prostaglandins improved GFR without an effect on RBF, suggesting a possible non-hemodynamic action of prostaglandins to limit injury. Because acute renal failure remains an important cause of patient morbidity and mortality, the search for effective agents to prevent or attenuate injury or to enhance recovery from acute renal insult continues.

Other cell types can also be protected by prostaglandins against noxious stimuli. Gastric mucosa is the best studied model. Exogenous prostaglandins protect gastric mucosa against injury produced by aspirin, ethanol, and bile salts [16–20]. This protection has been termed "cytoprotection" to imply a beneficial effect localized to the cell, independent of changes in organ blood flow. The precise mechanism of cytoprotection remains to be defined.

The purpose of the present study was to elucidate the beneficial effects of a prostaglandin E_1 analogue in two in vivo models of acute renal injury: ischemia and mercuric chloride administration. We subsequently sought to determine whether these beneficial effects could be demonstrated in an in vitro model of hypoxic injury so that hemodynamic and other factors could be eliminated. Such a finding would indicate cytoprotection of renal epithelial cells by prostaglandins.

Methods

Ischemic acute renal failure

Male Sprague-Dawley rats (Harlan, Madison, Wisconsin, USA) weighing 225 to 290 g were allowed free access to food and water until the time of study. Rats were anesthetized with sodium pentobarbital (60 mg/kg i.p.) and an orogastric tube (PE-100) was temporarily placed for administration of drugs. The PGE_1 analogue misoprostol ([±]methyl[11α,13E]- 11,16-dihydroxy-16-methyl-9-oxoprost-13-en-1-oate; provided by Searle Research and Development, Skokie, Illinois, USA) was initially dissolved in absolute ethanol and then diluted in sodium phosphate buffer to yield a solution containing 200 μg/ml. Control animals received 0.5 ml of the ethanol/phosphate buffer via the orogastric tube and experimental animals received misoprostol 333 μg/kg. Thirty minutes later, a midline abdominal incision was made and the right kidney was removed. The left kidney was exposed, the perirenal fat removed, and the left renal artery exposed. A non-traumatic vascular clamp was then placed across the renal artery for 40 minutes. After removal of the clamp, the animal was prepared for clearance studies. In other animals bilateral flank incisions were made to expose the kidneys. Right nephrectomy and left renal artery occlusion were performed as described. After removal of the renal artery clamp, the animals were sutured and allowed to recover for 24

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hours for clearance studies as described below. In a third group of animals the effect of multiple preischemic doses of misoprostol was tested. Animals received misoprostol (or buffer) at 0800 and 1800 on the day before ischemia as well as 30 minutes before ischemia. In these animals renal function was measured 24 hours after ischemia.

The effects of renal ischemia on renal function were assessed by measurement of inulin clearance (GFR) and inulin extraction to calculate renal blood flow. Animals were placed on a heated, temperature-controlled table and a 23-gauge needle was also inserted into the renal vein and secured with cyanoacrylate. A priming infusion of 10% inulin in saline was begun at 0.06 ml/min for 15 minutes, followed by a sustaining infusion of 0.02 ml/min for the duration of the study. After a 60-minute equilibration period, three 20-minute urine collections were made. Blood was obtained at the midpoint of each collection from the femoral artery and renal vein for plasma inulin determination. The concentration of inulin in urine and plasma was determined by the anthrone method and inulin clearance and renal plasma flow were calculated by the standard formulae. Renal blood flow was calculated from renal plasma flow and hematocrit by the standard formula.

Lipid peroxidation was measured after 40 minutes of ischemia plus 15 minutes of reperfusion, a time of maximum lipid peroxidation [21] by determining the renal cortical content of the lipid peroxidation product malondialdehyde (MDA) in a separate group of animals. MDA was measured by the method of Ohkawa, Ohishi and Yagi [22]. After ischemia plus reperfusion kidneys were rapidly removed and placed in iced phosphate buffered saline. Sections of renal cortex were suspended in a total volume of 3 ml 100 mM KCl plus 0.003 M EDTA and homogenized with a Polytron (Brinkman Instruments, Westbury, New York, USA) at setting 8 for 15 seconds. Homogenates were then centrifuged at 600 g for 10 minutes. Two hundred microliters of supernate were added to 0.2 ml 8.1% sodium dodecyl sulfate, 1.5 ml 20% acetic acid (pH 3.5), 1.5 ml 0.8% thiobarbituric acid and 6.6 ml water. This solution was heated to 95°C for 60 minutes. After addition of 1.0 ml water and 5.0 ml of an n-butanol/pyridine mixture (15:1 vol/vol) the mixture was vigorously shaken and centrifuged at 2000 g for 15 minutes. The absorbance of the upper organic layer at 532 nm was determined in a spectrophotometer. Absorbance of tissue samples was compared to results obtained using freshly prepared malonaldehyde tetraethylacetal standards (Sigma Chemical Co., St. Louis, Missouri, USA). MDA values were expressed per mg protein. All determinations of MDA were performed in duplicate.

Mercuric chloride-induced acute renal failure

Rats were briefly anesthetized with ether and then given misoprostol or buffer as described above. Thirty minutes later each animal was given mercuric chloride ($HgCl_2$) in a dose of 2 mg/kg subcutaneously as a 2.5 mg/ml 0.9% saline solution. Twenty-four hours later the animals were prepared for clearance studies and GFR and renal blood flow were measured as above. In a second set of studies the dose of $HgCl_2$ was decreased to 1.5 mg/kg to reduce the amount of renal injury.

In vitro studies of hypoxic renal cell injury

Cell culture. Rat renal proximal tubule segments were isolated by the method of Gesek, Wolff and Standhoy [23] which employs collagenase digestion of the renal cortex followed by Percoll density gradient centrifugation. This procedure yielded a preparation primarily consisting of proximal tubule fragments (>95%) with approximately 90% viability by vital dye exclusion. Culture medium was RPMI 1640 containing amino acids and vitamins, 11 mM glucose, 1 mM Ca $(NO_3)_2$, 5.4 mM KCl, 0.4 mM $MgSO_4$, 103 mM NaCl, 5.6 mM Na_2HPO_4, 23.8 mM $NaHCO_3$, 10 mM Hepes, 10% fetal calf serum, 100 mU/ml penicillin, and 100 mg/ml streptomycin to which 10 ng/ml epidermal growth factor (EGF), 5 mg/ml transferrin, 5 mg/ml insulin and 10^{-8} M dexamethasone (final concentrations) were added. Tubule fragments were suspended in culture medium and plated onto collagen gel-coated (Sigma, Type I) plastic 12-(4.5 cm^2) multiwell plates. Culture medium was changed every other day. Primary cultures were used for all studies. The proximal origin of cultured cells was supported by preliminary studies which showed expression of proximal tubule brush border enzymes and formation of domes, evidence of vectorial transport. Confluent monolayers were assayed in situ for the brush border enzyme alkaline phosphatase using a cytochemical assay as described by Wachsmuth and Torhorst [24]. Cells were fixed with formaldehyde-methanol and reacted with 0.33 mM naphthol AS-BI phosphate and 3 mM fast red violet LB (Sigma). Virtually 100% of cells showed staining for alkaline phosphatase. Cells were also assayed for gamma-glutamyltranspeptidase activity, another proximal tubule brush border marker, using L-gamma-glutamyl-3-carboxy-4-nitroanilide as the substrate while spectrophotometrically monitoring the reaction at 37°C [25]. Cell homogenates derived from sub-confluent monolayers contained 1.04 ± 0.05 μmol/min/mg protein of the enzyme ($N = 4$), whereas fresh renal cortex had an enzyme activity of 8.88 ± 0.43 μmol/min/mg protein.

Hypoxic cell injury. Cells were studied in a sub-confluent stage, usually four days after initial plating. Forty-eight hours before study the culture medium was switched to a glucose-free formulation of the usual culture medium to enhance cell susceptibility to hypoxia/reoxygenation injury [26]. Two hours before study, the medium was changed to glucose-free medium without added growth factors. Cell plates were placed in an airtight glass chamber under a continuous flow of humidified gas and maintained at 37°C: hypoxia (95% N_2, 5% CO_2) for 60 minutes followed by reoxygenation (95% O_2, 5% CO_2) for 30 minutes. Cell injury was quantitated as the percent of total cellular lactate dehydrogenase (LDH) released into the culture medium. LDH was measured in the incubation medium and in homogenized cells (Teflon-glass motorized mortar and pestle) by monitoring the reduction of NAD in the presence of lactate at 339 nm. The two values were summed to obtain total LDH.

To test the ability of misoprostol to protect against hypoxic injury in vitro, misoprostol (10^{-9} to 10^{-4} M) was added to the incubation medium two hours before the cells were subjected to hypoxia and reoxygenation. In other studies either prostacyclin, PGE_2, or arachidonate were similarly prepared and added to the incubation media prior to hypoxia.

The ability of misoprostol to protect against other causes of

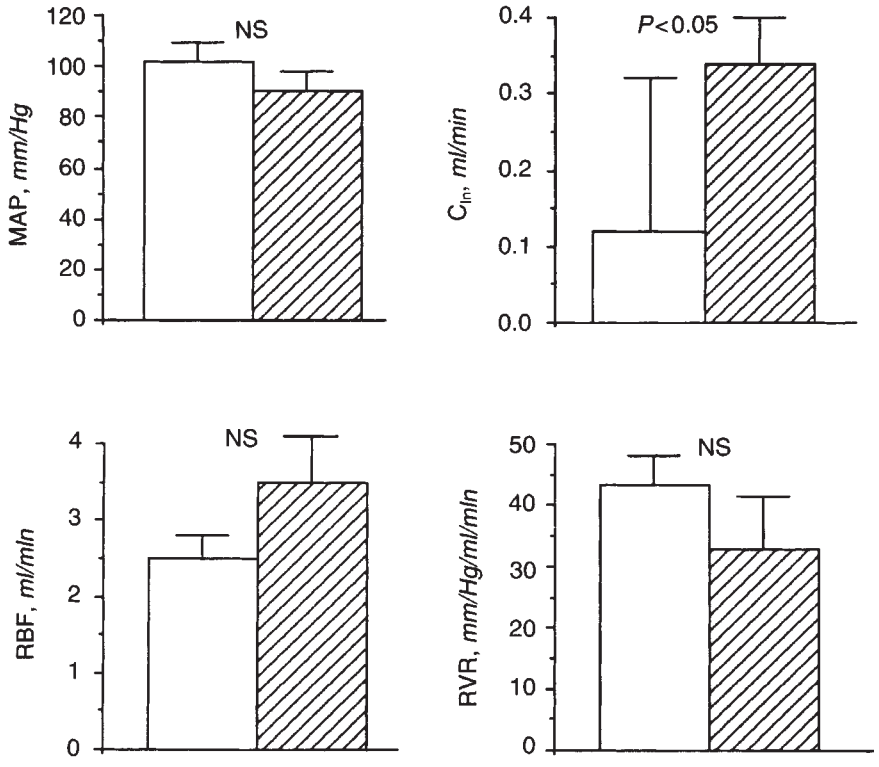

Fig. 1. *Effect of misoprostol on renal function one hour after ischemia.* Abbreviations are: MAP, mean arterial pressure; C_{in}, inulin clearance; RBF, renal blood flow; RVR, renal vascular resistance; NS, no significant difference. Symbols are: (□) control, $N = 6$; (▨) misoprostol, $N = 6$.

cell injury was tested by exposing cells to tert-butyl hydroperoxide, an oxidant whose effects on renal epithelial cells has been well characterized [27, 28]. Cells were exposed to tert-butyl hydroperoxide (2 to 10 mM) for 30 to 60 minutes without or with concomitant addition of misoprostol.

Electron microscopy. Cells were grown on microporous cell culture inserts (Falcon, Becton Dickinson Labware, Lincoln Park, New Jersey, USA) to facilitate sectioning. Cells were fixed in 2.5% phosphate-buffered glutaraldehyde for one hour, and post-fixed in 1.5% buffered osmium tetroxide solution for 30 minutes. They were dehydrated in graded alcohol, transferred to acetone, and embedded in resin (Poly/Bed 812, Polysciences, Inc., Warrington, Pennsylvania). Ultrathin sections were mounted on a copper grid and stained with uranyl acetate and lead citrate, and examined with a Philips 201 electron microscope.

Results are reported as mean ± standard error. Intergroup comparisons were made by Student's *t*-test for unpaired samples. When multiple comparisons were made the Bonferroni method was used to determine the critical values for *t*. For nonparametric data the Neuman-Keuls test was used.

Results

Ischemic renal injury

Pretreatment with misoprostol resulted in significant improvement in renal function whether measured one hour or 24 hours after ischemia. When animals were studied after one hour of reperfusion, those that had received misoprostol tended to have lower mean arterial pressure (90 ± 8 vs. 102 ± 7 mm Hg), but this difference was not significant. As seen in Figure 1, inulin clearance was higher in misoprostol-treated animals (0.34 ± 0.07 vs. 0.12 ± 0.02 ml/min) although renal blood flow and renal vascular resistance were similar in control and misoprostol-treated animals. Better renal function in misoprostol-treated animals was also reflected by a lower fractional excretion of sodium (4.6 ± 1.5 vs. 9.9 ± 2.1; $P < 0.05$) and a higher urine-to-plasma creatinine ratio (25.4 ± 5.4 vs. 9.9 ± 2.1; $P < 0.025$). In the control animals the relationship between RBF and GFR was described by the equation: GFR = 0.0309 × RBF + 0.0442. In misoprostol-treated animals the actual GFR, 0.34 ml/min, was much higher than the predicted value of 0.15 ml/min, suggesting that factors other than renal blood flow enhancement resulted in the higher GFR in these animals.

A separate group of animals studied 24 hours after ischemia demonstrated similar beneficial effects of prostaglandin pretreatment. Inulin clearance was threefold higher in misoprostol-treated rats (Fig. 2). In addition, fractional sodium excretion was lower (5.0 ± 1.0 vs. 11.8 ± 3.0%) in misoprostol-treated animals.

Multiple preischemic doses of prostaglandin E also provided protection against postischemic renal dysfunction. Twenty-four hours after ischemia misoprostol-treated animals had a GFR twice as high as control animals despite similar renal blood flow (Table 1). However, inulin clearance in animals receiving three preischemic doses of misoprostol was not significantly greater than that in animals receiving a single oral dose of misoprostol 30 minutes before ischemia.

These beneficial effects of prostaglandin E pretreatment on postischemic renal function did not appear to be due to any antioxidant activity of the prostaglandin. As shown in Figure 3, although renal cortical malondialdehyde content increased after ischemia and 15 minutes of reperfusion, there was no difference

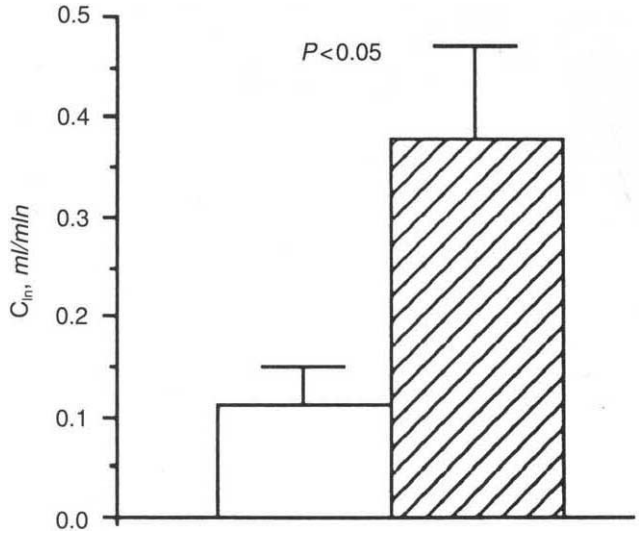

Fig. 2. *Effect of misoprostol on renal function 24 hours after ischemia.* Abbreviation is C_{in}, inulin clearance. Symbols are: (□) control, $N = 5$; (▨) misoprostol, $N = 6$.

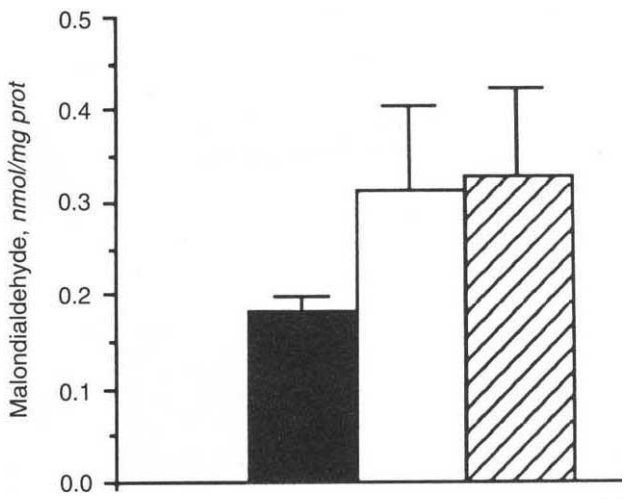

Fig. 3. *Lack of effect of misoprostol on free radical-mediated lipid peroxidation after renal ischemia.* Symbols are: (■) nonischemic, $N = 6$; (□) ischemia, $N = 6$; (▨) misoprostol + ischemia, $N = 6$.

Table 1. Effect of multiple preischemic doses of misoprostol on postischemic renal function

	MAP	GFR	RBF	V
	mm Hg	*ml/min*		*μl/min*
Control ($N = 6$)	104 ± 3	0.26 ± 0.08	5.4 ± 1.4	19.9 ± 4
Misoprostol ($N = 6$)	100 ± 4	0.55 ± 0.08	6.5 ± 0.7	17.4 ± 3
P value	NS	< 0.03	NS	NS

Abbreviations are: MAP, mean arterial pressure; GFR, glomerular filtration rate; RBF, renal blood flow; V, urinary flow rate; NS, no significant difference.

Table 2. Effect of misoprostol on $HgCl_2$-induced renal dysfunction

	MAP	GFR	RBF	V
	mm Hg	*ml/min*		*μl/min*
$HgCl_2$, 2.0 mg/kg				
Control ($N = 7$)	118 ± 4	0.06 ± 0.02	0.63 ± 0.2	3.8 ± 1.4
Misoprostol ($N = 7$)	117 ± 4	0.14 ± 0.04	1.6 ± 0.3	8.0 ± 2.0
P value	NS	< 0.05	< 0.05	NS
$HgCl_2$, 1.5 mg/kg				
Control ($N = 8$)	127 ± 5	0.35 ± 0.08	4.1 ± 1.0	4.1 ± 0.5
Misoprostol ($N = 5$)	117 ± 5	0.81 ± 0.14	6.0 ± 0.8	4.2 ± 0.9
P value	NS	< 0.02	NS	NS

between buffer-treated and misoprostol-treated postischemic kidneys. Thus, misoprostol did not appear to limit free radical-mediated lipid peroxidation after ischemia.

Toxic renal injury

Misoprostol was also protective against a form of toxic acute renal failure, that caused by mercuric chloride administration. Table 2 demonstrates the dose dependency of renal injury in this model. Control animals receiving 2 mg/kg $HgCl_2$ had an inulin clearance of 0.06 ml/min, whereas those that received 1.5 mg/kg had an inulin clearance of 0.35 ml/min. For each dose of $HgCl_2$ misoprostol resulted in a more than twofold increase in inulin clearance. This increase in GFR by misoprostol was not consistently accompanied by an increase in renal blood flow. Thus, these findings are quite similar to those in renal ischemia.

Renal hypoxic injury in vitro

When primary cultures of proximal tubule epithelial cells were subjected to 60 minutes of hypoxia and 30 minutes of reoxygenation they suffered considerable injury. LDH release, used as a measure of critical injury causing loss of intracellular constituents, averaged 36.2 ± 2.5% of total LDH with this insult. Misoprostol prevented or greatly attenuated this injury. Figure 4 shows the compiled data for the six sets of studies employing 10^{-7} to 10^{-6} M misoprostol. Misoprostol reduced LDH release (injury) by 71% ($P < 0.001$). The dose response to misoprostol in two sets of studies of hypoxic cell injury showed a biphasic response (Fig. 5) with concentrations less than 10^{-8} M showing no beneficial effect and concentrations of 10^{-4} M having a slightly detrimental effect. Misoprostol had no adverse effect on LDH release in cells not subjected to hypoxia and reoxygenation, even in concentrations of 10^{-4} M (Table 3).

The protective effects of prostaglandins in this type of acute injury was not confined to the E class of prostaglandins. Although misoprostol and PGE_2 provided significant protection against hypoxic renal cell injury in vitro, so too did prostacyclin (Table 4). The effects of arachidonic acid did not reach statistical significance. On the other hand, misoprostol was not protective against injury produced by an exogenous oxidant, such as tert-butyl hydroperoxide (Table 5).

Transmission electron microscopic evaluation was also revealing. Cells studied under normoxic conditions were polarized (apical microvilli, basal intermediate filaments, tight junctions with interdigitation of lateral membranes) and had intracellular organelles consistent with actively proliferating, metabolically active cells (prominent nucleoli, endoplasmic

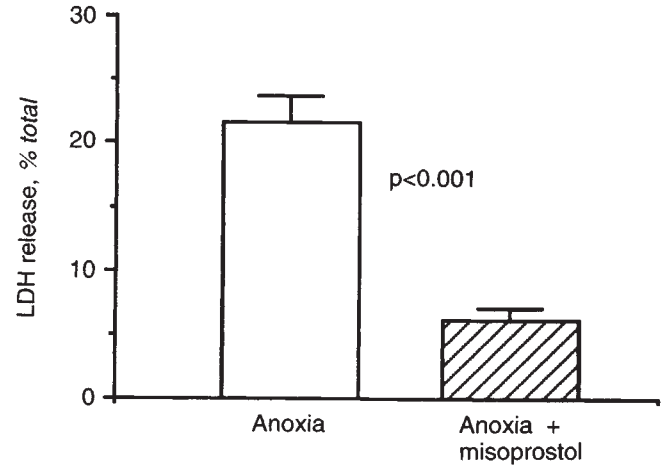

Fig. 4. *Effect of misoprostol on hypoxia/reoxygenation injury of renal tubule epithelial cells, measured as lactate dehydrogenase (LDH) release.* For this figure basal, LDH release for non-ischemic cells from each batch of cells (range 8.5 to 28.8%) was subtracted from the ischemic values to facilitate comparison of the data from six separate trials.

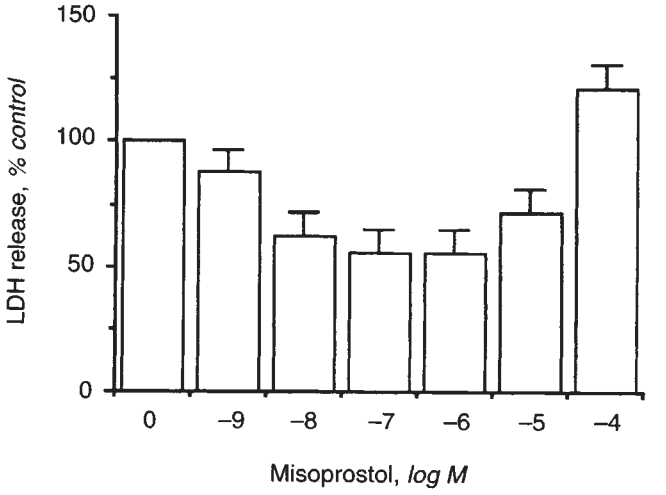

Fig. 5. *Dose response relationship of misoprostol protection of hypoxic renal tubule epithelial cells.* Data was obtained from two sets of studies each employing four cell wells for each dose of misoprostol tested. Misoprostol 10^{-6} to 10^{-8} was significantly protective against hypoxic renal injury ($P < 0.05$ vs. control by Student's *t*-test with Bonferroni's correction).

reticulum, golgi apparatus, and polyribosomes; Fig. 6). Cells studied after hypoxia and reoxygenation in the absence of misoprostol revealed numerous signs of both reversible and irreversible injury. About 30% of the cells could be considered dead, with extensive disruption of the plasma membrane, swelling and disruption of mitochondria, disintegrating nuclei, and cell detachment (Fig. 7). Other cells showed more limited disruption of the apical and mitochondrial membranes, although these changes are regarded as signs of irreversible injury (Fig. 8). All cells showed complete or nearly complete loss of apical microvilli. Several cells had large apical membrane blebs, fluid accumulation in the cytoplasm, and dilatation of the endoplasmic reticulum. In addition, they all had condensation of their

Table 3. Effect of misoprostol on LDH release in cells not subjected to hypoxia and reoxygenation

	Concentration ($\times 10^X$ M)	LDH release %
Control ($N = 7$)	0	19.2 ± 1.1
Misoprostol ($N = 4$)	−6	13.0 ± 0.7[a]
Misoprostol ($N = 4$)	−4	9.3 ± 0.8[a]
Control ($N = 8$)	0	8.5 ± 1.2
Misoprostol ($N = 8$)	−7	8.9 ± 0.6

Cells were exposed to the indicated concentration of misoprostol for 90 minutes to determine whether the prostaglandin analogue had any effect on basal LDH release.

[a] $P < 0.05$ vs. control

Table 4. Effect of several prostaglandins and arachidonic acid on hypoxic tubule epithelial cell injury

Condition	LDH release %
Hypoxia/reoxygenation	24.2 ± 3.3
+ misoprostol (10^{-6} M)	4.2 ± 1.3[a]
+ prostacyclin (10^{-6} M)	4.4 ± 1.8[a]
+ PGE_2 (10^{-6} M)	1.4 ± 0.7[a]
+ arachidonic acid (10^{-6} M)	11.8 ± 3.2

To facilitate comparison of data from three separate trials, the values for LDH release are expressed as specific LDH release (that due to hypoxia/reoxygenation), obtained by subtracting the basal LDH release for non-hypoxic cells from each batch from the hypoxic value. The basal LDH release ranged from 10.7 to 21.7% for these cells.

[a] $P < 0.05$ vs. hypoxia/reoxygenation by Student's *t*-test with Bonferroni's correction

Table 5. Lack of effect of misoprostol (10^{-6} M) on tert-butyl hydroperoxide-induced renal tubule epithelial cell injury

	t-butyl hydroperoxide *mM*	Duration exposure *min*	LDH release %
Control cells	2	30	5.1 ± 1.0
Misoprostol-treated cells	2	30	5.1 ± 0.6
Control cells	4	30	6.9 ± 0.9
Misoprostol-treated cells	4	30	10.5 ± 0.8[a]
Control cells	2	60	21.7 ± 1.1
Misoprostol-treated cells	2	60	24.5 ± 0.6[a]
Control cells	4	60	21.6 ± 1.6
Misoprostol-treated cells	4	60	26.3 ± 0.8[a]
Control cells	10	60	52.3 ± 2.2
Misoprostol-treated cells	10	60	56.6 ± 2.7

[a] $P < 0.05$ vs. control

chromatin and multiple intracellular autophagosomes (at various stages of development), changes reflecting reversible injury. Two-thirds of the cells appeared to have lost their polarity (loss of apical microvilli, basal intermediate filaments, and junctions between adjacent cells). Cells treated with misoprostol and exposed to hypoxia and reoxygenation were quite different in appearance. Despite hypoxia and reoxygenation every cell had the usual quantity of microvilli, no disruption of the apical (or basolateral) membrane, and preservation of cell

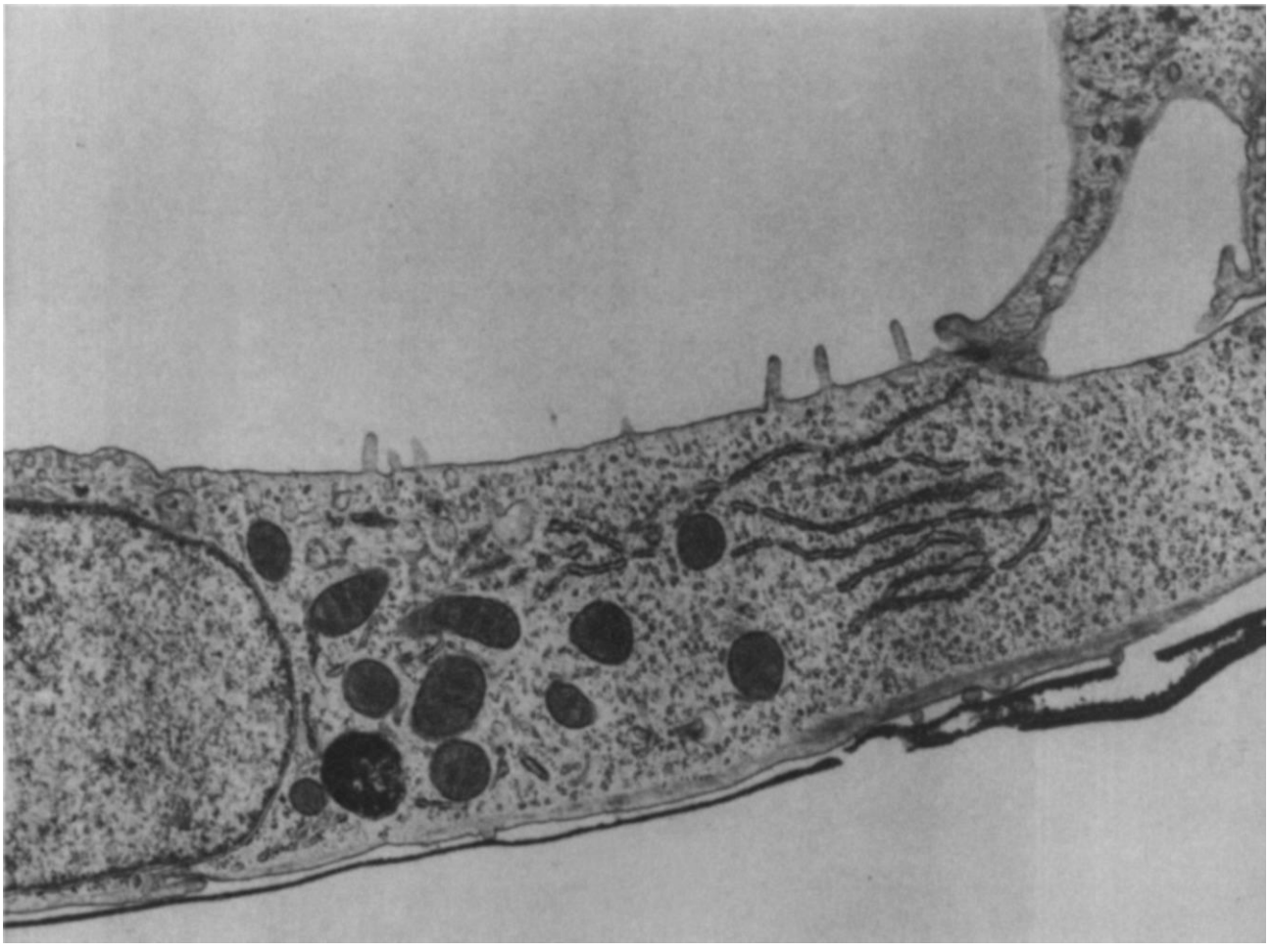

Fig. 6. *Epithelial cells of proximal tubule origin cultured under normoxic conditions.* Note the apical microvilli, well-developed rough endoplasmic reticulum, numerous polyribosomes, normal mitochondria, and basal collection of intermediate filaments. Also seen are an autophagosome (next to the nucleus) and a tight junction with a process from an adjacent cell. All cell membranes are intact. The dark material beneath the cell is the collagen gel (uranyl acetate-lead citrate × 10,758).

polarity (Fig. 9). Nevertheless, these cells did contain numerous autophagosomes, condensed chromatin, and even occasional examples of mitochondrial membrane disruption.

Discussion

The ability of prostaglandins to protect against ischemic injury of the gut, myocardium, and kidneys has been known for several years [2–13, 16, 29]. Previous studies of acute renal injury have demonstrated a beneficial effect of prostaglandins in some but not all studies. Moskowitz, Korobkin and Rambo found that prostaglandin E_1 increased renal blood flow, but not GFR, when given to dogs several days after renal ischemia [2]. When Mauk and colleagues gave prostaglandin E_2 by intrarenal infusion prior to norepinephrine-induced renal ischemia, protection of GFR was noted [3]. Mundy et al and Neumayer et al found that prostacyclin also protected against ischemic renal injury in the dog. In the latter study the beneficial effects of prostacyclin were felt to be secondary to renal vasodilatation [4, 5]. Tobimatsu et al also observed protection against ischemic renal dysfunction in dogs given prostaglandin E_1 [6]. They observed substantial increases in renal blood flow when prostaglandins were given. However, Casey et al could not demonstrate a beneficial effect of either prostaglandin E_1 or prostacyclin on renal function in dogs subjected to renal ischemia, although renal histology was improved by prostaglandin administration [7]. Several other investigators have also reported, in less detail, beneficial effects of prostaglandins in renal ischemia [8–11]. More recently, both Lipschitz and Barnes, and Finn, Hak and Grossman demonstrated intravenous prostacyclin to protect against renal ischemic injury in the rat [12, 13]. In neither study was a mechanism for this protective effect found, but Finn and coworkers suggested that a hemodynamic effect was not likely to be the explanation because total renal blood flow was not increased in their prostacyclin-treated animals. Prostaglandin E also protected rats against glycerol-induced acute renal failure, but did not protect dogs against uranyl nitrate-induced injury [3, 14].

The present study confirms a beneficial effect of prostaglandin E to protect against ischemic renal injury in the rat. The magnitude of this effect was similar to that previously demonstrated for free radical scavengers, adenosine triphosphate, or mannitol. We also found protection in toxic acute

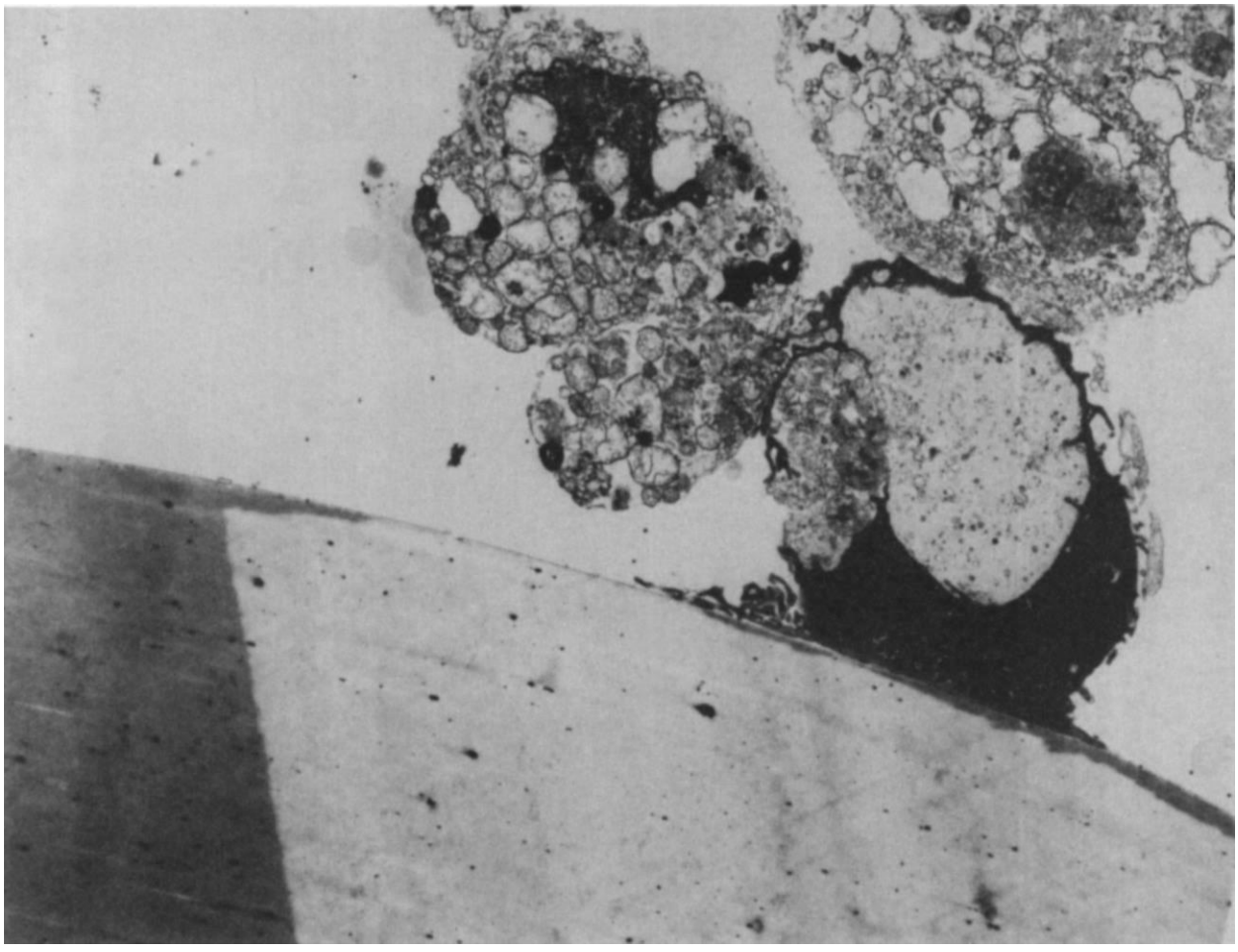

Fig. 7. *After hypoxia and reoxygenation, in the absence of misoprostol, cells show detachment, extensive disruption of plasma membranes, swelling and disruption of mitochondria, and nuclear disintegration (uranyl acetate-lead citrate × 5943).*

renal failure caused by mercuric chloride. These studies were not designed to determine the mechanism of such an effect. The lack of marked increases in renal blood flow might suggest that hemodynamic factors were not primarily responsible for renal protection. However, a beneficial redistribution of renal blood flow or maintenance of microvascular integrity cannot be ruled out by the in vivo studies. Furthermore, we evaluated renal blood flow at only one time point and there was a trend towards higher values. The strongest argument against hemodynamic effects being solely or predominantly responsible for the beneficial effects of misoprostol is the finding of protection in vitro. Certainly hemodynamic factors could be additive to other cytoprotective mechanisms.

The dose of misoprostol used was one we previously showed to have no effect on GFR, renal blood flow, or solute excretion in normal rats, although normal rats do sustain a small decrease in blood pressure with similar doses [30]. If anything, the hypotensive effect of misoprostol would oppose any beneficial effects in renal ischemic injury. The present studies are also important because a single dose of an orally active prostaglandin, rather than a continuous intravenous or intra-renal infusion, was protective. The clinical relevance of this finding is apparent, especially since misoprostol has been safely given to patients for other indications.

The ability of prostaglandins to protect gastric mucosa against a variety of noxious stimuli suggests a mechanism of action involving a final common pathway for tissue injury. In a variety of studies exogenous or endogenous prostaglandins protected against injury caused by aspirin, ethanol, bile salts, and hydrochloric acid [16]. Prevention of gastric mucosal barrier disruption, stimulation of mucus secretion, stimulation of non-parietal cell alkaline secretion, enhancement of mucosal blood flow, and preservation of microvascular integrity have been hypothesized to be the responsible mechanism [16, 17]. At the cellular level, stimulation of DNA and RNA synthesis, stimulation of cellular transport processes, stimulation of cyclic AMP formation, stabilization of tissue lysosomes, maintenance of sulfhydryl content, and stimulation of surface-active phospholipids have been postulated to produce these benefits [16, 18–20]. In ischemic myocardial tissue prostaglandins have been clearly demonstrated to inhibit infiltration of the postischemic muscle by neutrophils and thus limit injury [29].

In the kidney the first three possibilities suggested for gastric mucosa protection are not applicable. Of the others, effects on

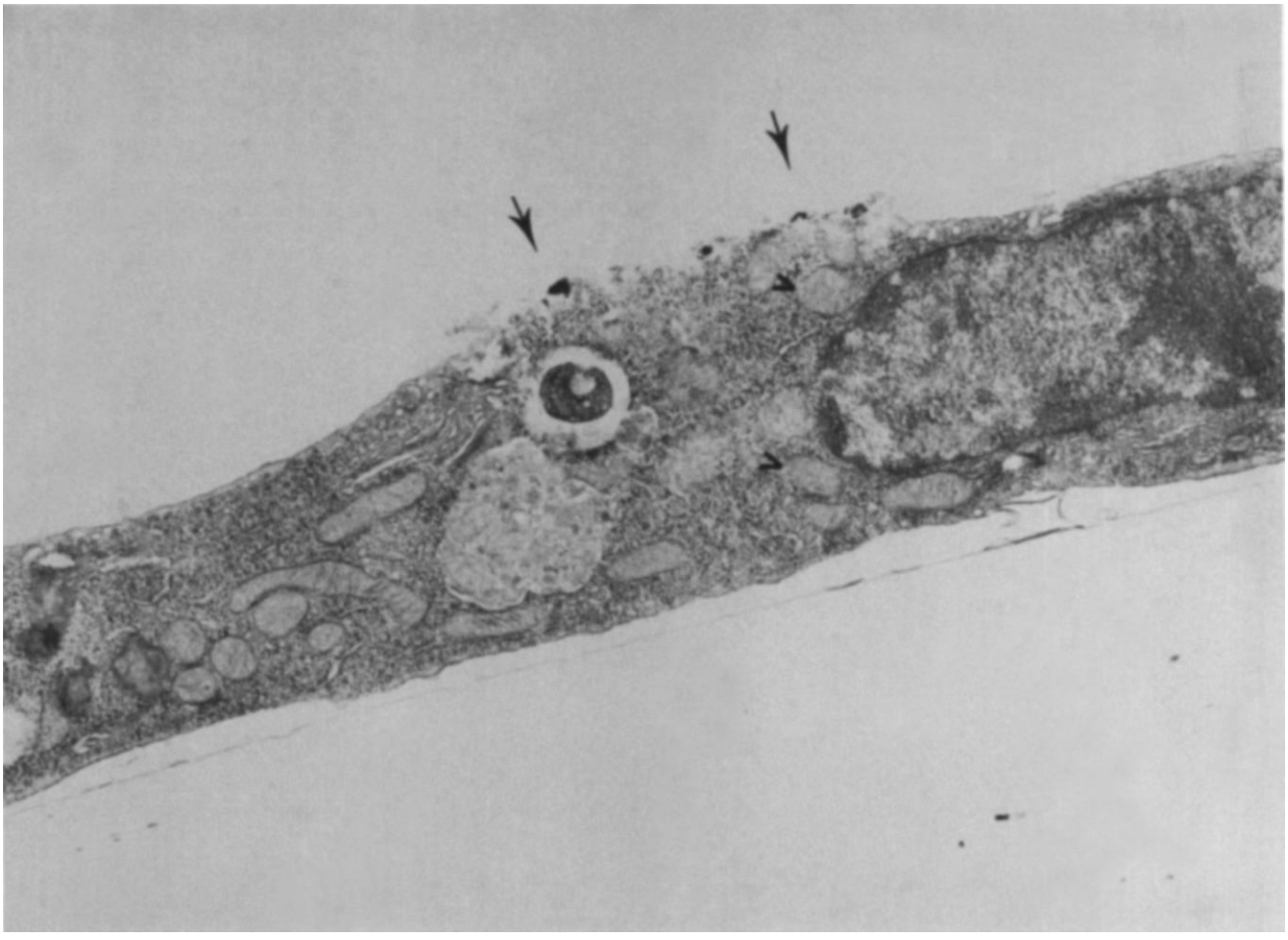

Fig. 8. *After hypoxia and reoxygenation, in the absence of misoprostol, this cell shows less damage than those in Figure 7.* Nevertheless, disruption of the apical membrane (arrows) and mitochondrial membranes (arrowheads) are features of irreversible injury (uranyl acetate-lead citrate × 16,394).

renal blood flow or microvascular integrity can be excluded because prostaglandins protected against hypoxic injury in an in vitro system. Similarly, invading neutrophils are irrelevant in the in vitro model. Although these studies clearly demonstrate for the first time a cytoprotective effect of prostaglandins for renal epithelial cells, the precise mechanism for this effect in the kidney as well as in other tissues remains unclear. These findings do suggest that prostaglandin-mediated cytoprotection may be a generalized phenomenon.

The histologic studies do provide clues as to the site of protection provided by prostaglandins. Misoprostol-treated cells had preservation of the microvilli and intact apical membranes unlike control cells subjected to hypoxia and reoxygenation in which these changes were extensive. Disruption of the apical membrane, an irreversible change, was not seen in any misoprostol-treated cell. Misoprostol-treated cells did show many changes associated with reversible injury, including chromatin condensation and formation of autophagosomes. Therefore, prostaglandins had a greater protective effect on surface membranes than on intracellular membranes.

Both the in vivo and the in vitro studies suggest, however, that prostaglandins do not act by preventing oxygen free radical formation or the injurious effects of reactive oxygen species. Although oxygen free radicals are important mediators of ischemic injury in vivo and in vitro, they are probably not important in mercuric chloride-induced acute renal failure [21, 26, 31]. Misoprostol protected in all of these settings. In the present study although ischemia caused the expected increase in free radical-mediated lipid peroxidation in the kidney, misoprostol did not lessen kidney lipid peroxidation [21]. Finally, misoprostol was ineffective in preventing oxidant injury of renal tubule epithelial cells caused by tert-butyl hydroperoxide. Thus, in the aggregate these results exclude one potential mechanism for prostaglandin cytoprotection.

One is tempted to further speculate as to potential mechanisms of prostaglandin cytoprotection. Prostaglandin E_2 stimulates adenylate cyclase in the proximal tubule [32]. Cyclic AMP could then induce protein phosphorylation via cAMP-dependent protein kinase or inhibit the Na-H antiporter [33]. However, it is widely believed that prostaglandins have no direct effect on proximal tubule ion transport [34] and there is no evidence to support the former possibility. Finally, PGE_1 has been observed to increase membrane fluidity in synaptosomal membrane vesicles in concentrations similar to those employed

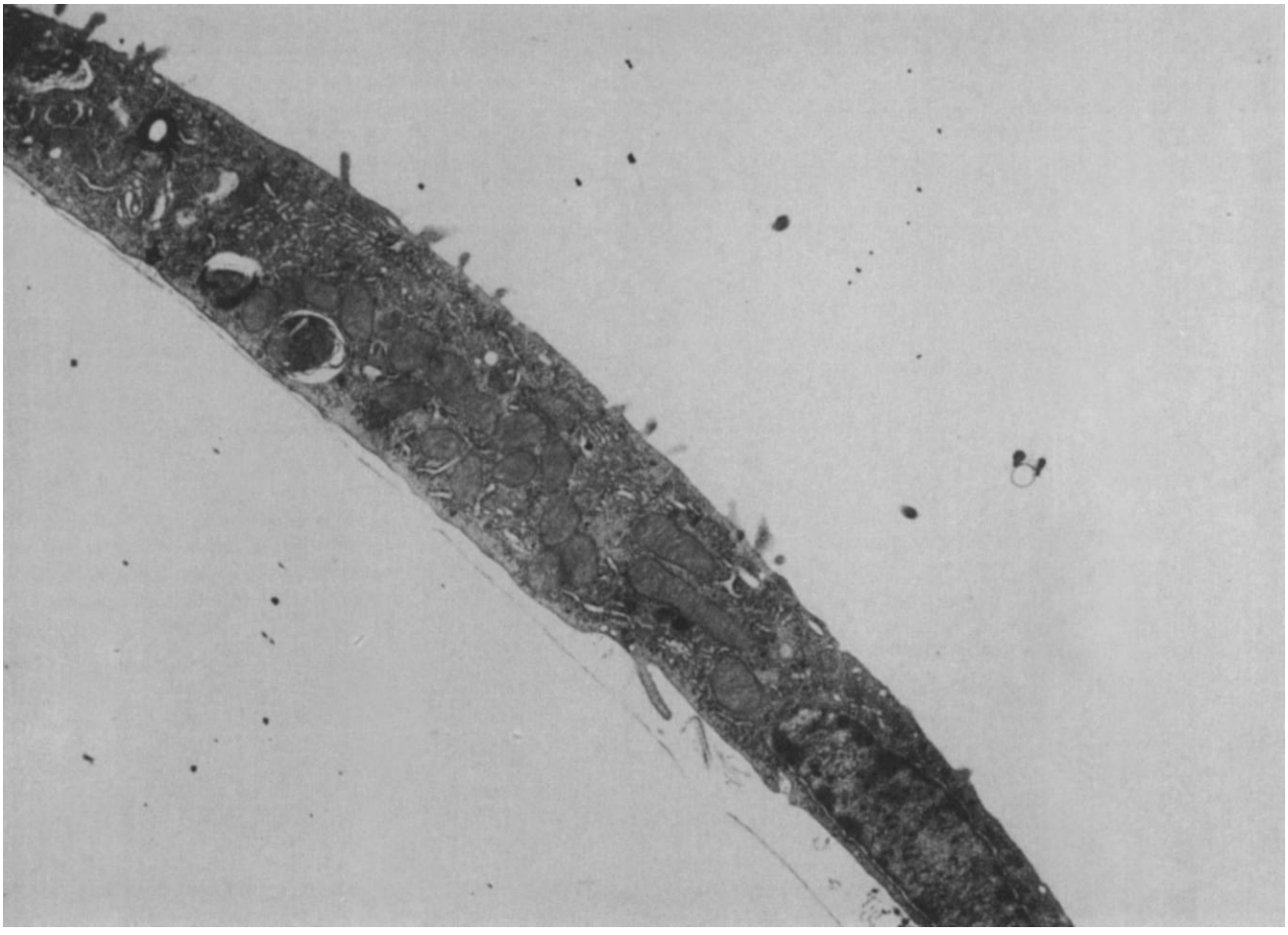

Fig. 9. *Cell treated with misoprostol prior to exposure to hypoxia and reoxygenation.* Note the preservation of apical microvilli and integrity of membranes. Autophagosomes and chromatin condensation represent reversible injury (uranyl acetate-lead citrate × 14,710).

here (10^{-6} M) [35]. Although increased membrane fluidity could enhance the activity of membrane-bound enzymes and transmembrane ion transporters, it is not clear how such an effect would be protective during acute cellular injury. Thus, considerable additional work will be necessary to explain this important effect of prostaglandins on renal proximal epithelial cells.

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